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MUTAGENIC RESIDUES RECOVERED FROM GRANULAR ACTIVATED CARBON AFTER ITS USE IN DRINKING WATER TREATMENT

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16. ABSTRACT

In addition to its use in removal of compounds which cause taste, odor or color problems in drinking water, granular activated carbon (GAC) has received increasing attention as a treatment for removal of compounds known or suspected to be of toxicological significance. An experimental full scale GAC system for such water treatment has been developed at the Cincinnati Water Works. We have recently described the results of a study which examined this system throughout the adsoptive life of the GAC, monitoring trends both in the production of nonvolatile mutagens by chlorine disinfection and in the removal of such bioactive compounds from the water. Mutagenicity values were determined with Salmonella tester strains in assays of water residue organics extracted using XAD-2. None of the samples of settled unchlorinated water was mutagenically active in that study, while such samples were consistently active following chlorination. Mutagenic activity in chlorinated water was effectively removed by GAC treatment throughout that study, even after the removal of total organic carbon (TOC) had reached a steady level of about 0.65 that contained in the water influent to the column. We then demonstrated that residues extracted from the used GAC were mutagenic. Differences in that activity were shown for residues recovered from the top, middle or bottom of the used CAG adsorber. This paper presents further analysis of such GAC extracted organics. The mutagens can be separated into two populations, one of which contains relatively non polar compounds and shows mutagenic activity similar to that seen among other residues from this drinking water source, whether extracted by us using XAD resins, or by others using reverse osmosis. In the other population more polar compounds predominate, including compounds and mutagenic activity which are poorly recovered using our XAD-2/XAD-7 resin procedure.

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INTRODUCTION

In addition to its use in removal of compounds which cause taste, odor or color problems in drinking water, granular activated carbon (GAC) has received increasing attention as a treatment for removal of compounds known or suspected to be of toxicological significance (1-3). An experimental full scale GAC system for such water treatment has been developed at the Cincinnati Water Works (4,5). We have recently described the results of a study which examined this system throughout the adsorptive life of the GAC, monitoring trends both in the production of non-volatile mutagens by chlorine disinfection and in the removal of such bioactive compounds from the water (6). Mutagenicity values were determined with Salmonella tester strains in assays of water residue organics extracted using XAD-2. None of the samples of settled unchlorinated water was mutagenically active in that study, while such samples were consistently active following chlorination. Mutagenic activity in chlorinated water was effectively removed by GAC treatment throughout that study, even after the removal of total organic carbon (TOC) had reached a steady state level of about 0.65 that contained in the water influent to the column.

We then demonstrated that residues extracted from the used GAC were mutagenic. Differences in that activity were shown for residues recovered from the top, middle or bottom of the used GAC adsorber. The data presented here in Figure 1 are reproduced from our earlier publication of that work (6).

This paper presents further analyses of such GAC extracted organics. The mutagens can be separated into two populations, one of which contains relatively non polar compounds and shows mutagenic activity similar to that seen among other residues from this drinking water source, whether extracted by us using XAD resins, or by others using reverse osmosis. In the other population more polar compounds predominate, including compounds and mutagenic activity which are poorly recovered using our XAD-2/XAD-7 resin procedure.

MATERIALS AND METHODS

Solvents were nanograde (Mallinckrodt), HPLC grade (Fisher Scientific) or distilled-in-glass (Burdick and Jackson). Water of Type I quality was prepared by the use of a water conditioning system (Continental) as previously described (7). Other chemicals were purchased as reagent grade from commercial sources and were used as obtained. Reagent XAD-2 and XAD-7 resins prepared according to USEPA criteria (8) were obtained from the Applied Science Division of the Milton-Roy Company. Resins were stored until use in amber bottles under methanol, and columns were formed by gravity from slurries of the same solvent as described (6). Columns were eluted using 4 column volumes of an 85:15 (vol:vol) mixture of hexane-acetone based on a method of LeBel et al. (9).

HPLC Separation. High performance liquid chromatography (HPLC) separations were performed on a Waters Associates Model ALC/GPC 204 with components as described previously (7). The unit was fitted with a Water Associates radial compression module column system which used 8 mm by 10 cm columns. For reverse phase HPLC separations, a stationary phase of 10 um silica particles bonded with octadecylsilane was utilized. A stationary phase of 10 um silica was used for normal phase separations. Operating conditions for this HPLC system were as described previously (7).

Analytical scale (ug level) reverse phase separations were accomplished using a linear solvent gradient from water to acetonitrile, (7). Preparative scale (mg level) normal phase HPLC separations were accomplished by the series of isocratic and linear gradient elutions as follows: 100% hexane for 2 min then a 2 min linear gradient to 80% hexane: 20% methylene chloride, Fraction 1; isocratic for 5 min then a 3 min linear gradient to 30% hexane: 70% methylene chloride, Fraction 2; isocratic for 2 min then a 2 min linear gradient to 100% methylene chloride , Fraction 3; isocratic for 5 min then a 5 min linear gradient to 100% methanol, Fraction 4; isocratic for 5 min or until all 254 nm absorbing components eluted, Fraction 5. Each HPLC fraction was gently evaporated at 35°C under a

stream of dry nitrogen, then the residue was dissolved in dimethyl sulfoxide for mutagenesis testing.

Mutagenicity Assay. The Salmonella mammalian microsome mutagenicity assay using strains TA98 and TA100 was based upon the method as described by Ames et al. (10) with strain verification tests and minor assay modifications as described by Loper et al. (6).

GAC used in water treatment. A schematic diagram of the California Plant of the Cincinnati Water Works and of the GAC treatment procedure is presented in Figure 2, and specific features of the adsorber are summarized in Table I; further details of water treatment procedures are available in reference 6. One GAC adsorber filled with freshly regenerated carbon was used to treat sand filtered chlorinated water at one million gallons per day for nine months as described (6).

Extraction of GAC. This process is described in more detail elsewhere (6). Samples of the carbon were taken from the adsorber prior to its use and at the end of nine months. Aliquots of the damp carbon samples were soxhlet extracted with methylene chloride for 24 hr, the extracts were passed through anhydrous sodium sulfate to remove any water and residues were concentrated by rotary evaporation. Some studies examined residues prepared in the same manner from carbon which had been removed from a separate GAC adsorber used several months earlier.

RESULTS

Figure 1 summarizes the diversity of mutagenic properties which we had observed earlier for residues extracted from the used GAC(6). We were interested in further investigation of the strain TA100 mutagens. Unlike the case with mutagenic activity for TA98, residues mutagenic for TA100 were recovered for carbon from all three levels of the adsorber. Thus mutagens for this strain were the most likely to break through the GAC adsorber into the water supply.

The first experiments were designed to test how well our XAD adsorption procedure could extract these compounds. Residues extracted from used carbon obtained from the top and the bottom of the GAC adsorber were dissolved in small volumes of acetone and then diluted into separate volumes of type I water to form solutions of 2.5 mg./l. These water samples were processed using separate sets of columns of XAD-2 and XAD-7 in sequence, and the residues were examined by reverse phase high performance liquid chromatography (RP-HPLC) and by mutagenicity assay using TA100.

Figure 3A shows 254 nm absorption chromatographic elution profiles for two control solutions, a solvent blank and the residue extracted from 350 mg of carbon removed from the top of the regenerated GAC adsorber at the start of the nine month experiment. Few peaks were observed for either of these controls. By contrast the chromatograms in Figure 3B show the results when the same RP-HPLC procedure was applied to residues extracted from carbon removed from the top or bottom of the used GAC adsorber. The "Top" and "Bottom" chromatographic elution patterns are for residues from 35 and 23 mg of carbon respectively. Numerous 254 nm absorbing components were separated over a wide range of polarity for each sample; however relative to the top carbon sample, the bulk of the absorbing components for the bottom sample were eluted earlier by more polar portions of the gradient.

This relatively polar material was poorly recovered from reconstituted aqueous samples using XAD-2 and XAD-7. The RP-HPLC chromatograms presented in Figure 3C are for residues recovered by XAD-2. Although some loss of 254 nm absorbing material was apparent for the Top sample, the major losses occurred for the polar portions of the Bottom sample, as may be seen by comparing the chromatograms in Figures 3B and 3C. Extracts from the XAD-7 columns yielded negligible amounts of residue which revealed only trace levels of 254 nm absorbing components when examined by RP-HPLC (data not shown).

A qualitatively similar effect was seen when these residues recovered by XAD were examined for mutagenic activity. As summarized in Figure 4, appreciable activity was lost from each residue by this procedure, but the loss was most pronounced for the residue sample originally obtained from the bottom of the GAC. The XAD-2 column recovered 22% of this mutagenic activity vs 37% of that contained in the residue from the Top carbon. No additional mutagenic activity was recovered by the column of XAD-7 used in tandem (Figure 4), even though this resin was included on the prospect that it might extract compounds more polar than those absorbed by XAD-2.

In a parallel study of drinking water organics from the same Water Works, we have examined similar residues for the mutagenic properties of subfractions obtained using preparative scale HPLC (Tabor et al., manuscript in preparation). For this the residues were extracted from carbon of another GAC absorber which earlier had been used extensively for water treatment. Figure 5 presents a 254 nm absorption chromatographic elution profile for a sample of parent residue separated by normal phase HPLC. Mutagenic activity was detected in subfractions containing nonpolar compounds which were eluted by hexane near the beginning of the HPLC separation, and in subfractions containing polar compounds which were eluted by methanol at the end of the separation. These mutagenic fractions were combined into Fractions 1 and 5 as depicted in the figure.

These two populations of mutagens shared several properties with those recovered from carbon obtained from the top and bottom of the other GAC absorber. In particular,

the mutagenic activity of Fraction 5 showed little or no recovery by XAD-2/XAD-7, when that Fraction was reextracted by the XAD procedure used earlier to obtain the data of Figure 4. A further experiment tested whether this relatively polar mutagenic material in Fraction 5 could be reextracted by XAD-2/XAD-7 from water solution at pH2. Control experiments showed this activity was stable to acidification and reneutralization. Nevertheless, no mutagenic activity was recovered by this XAD resin procedure when the Fraction 5 residue was redissolved in water at pH2, or at pH6, or when the effluent from the tandem columns for the pH6 sample was then acidified to pH2 and reextracted by an additional set of XAD columns (data not shown).

DISCUSSION

Although major progress has been made in recent years as to the origin and nature of mutagens in drinking water, including those mutagens which result from chlorination (11), it is nevertheless apparent that identified chemicals account for only a small portion of drinking water organics (11, see also 12 for a related study). These unknown mutagenic compounds are present in concentrations of 1 ug/liter or less among a variety of other chemicals, so that analyses for bioassay and compound identification require access to residue concentrates from large water samples. Recovery of such residue mixtures has relied principally upon reverse osmosis techniques or upon adsorption onto XAD resins (13). Based upon extensive analysis as summarized recently in reference 1, GAC is considered less reliable as a general method for representative recovery of extracted compounds, but rather is a preferred method for the direct removal of water contaminants.

Thus, for the experimental full scale GAC tratment system at the Cincinnati Water Works, evaluation of the removal of nonvolatile mutageins has involved the collection of water samples influent and effluent to the GAC and then the extraction of residues from these samples by these other methods. In a preliminary test of this GAC system using

reverse osmosis, Monarca et al(14) indicated that the GAC efficiently and preferentially removed mutagenic activity from the water. Our more recent published study supported and extended those conclusions. Using XAD-2 extracted residues to monitor GAC performance, we showed that this GAC treatment system continued to be effective for removal of Salmonella mutagens long after removal of total organic carbon reached a steady state (6). Compared to the results of Monarca et al using reverse osmosis, our use of XAD was more sensitive in detecting mutagen levels, expressed in levels of activity/liter equivalent dose.

However, based upon the studies reported in this paper, there is one class of relatively polar mutagens recoverable from GAC used in water treatment which is less capable of being absorbed and recovered using XAD-2/XAD-7. These mutagens are active for strain TA100 assayed -S9. They were recovered from the bottom carbon of the used GAC adsorber and probably are also represented in the mutagens among the relatively polar compounds of Fraction 5, a fraction obtained upon normal phase HPLC separation of residues extracted from a random aliquot of carbon taken from a holding tank containing previously used GAC. The mutagenic activity in Fraction 5 was not reextracted from reconstituted water solutions by XAD-2/XAD-7, whether these solutions were at pH6 or pH2. Thus these mutagens differ from a specific class of mutagens reported to be present in some drinking water sources, which does not absorb to XAD reins from drinking water but does from drinking water solutions acidified to pH2(3).

We have not established whether this class of TA100 mutagens was present in the chlorinated water influent to the GAC, or was formed on the GAC during the several months that the adsorber was in use. Although the influent water contained an average 2 ppm of chlorine, low levels of bacteria were detected in the effluent from this GAC system prior to rechlorination (J. DeMarco, personal communcation), so some biotransformation was possible. Nevertheless, since this class of mutagens was recovered from carbon taken from the bottom of the GAC adsorber at a level just above the effluent, these mutagens might be expected eventually to be desorbed into the drinking water supply. In that case, the

effective adsorptive life of a GAC treatment column for waterborne mutagens could best be monitored using GAC itself. Modest volumes of carbon at the bottom of the GAC adsorber could be removed periodically and then extracted using methylene chloride (6) for bioassay of strain TA100 mutagens. In the meantime, as is indicated by the data in Figure 5, residues extractable from full scale GAC absorbers provide a ready souce of residue organic mixtures for analyses of different chemical classes of waterborne mutagens.

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REFERENCES

1. National Academy of Sciences-National Research Council. 1980. An evaluation for activated carbon, in Drinking water and Health, Vol. 2 (National Academy Press, Washiongton, DC) pp. 251-380.
2. Rook, J.J. 1983. Comparison of removal of halogenated and other organic compounds by six types of carbon in pilot filters, in Treatment of Water by Granular Activated Carbon, Eds., M.J. McGuire and I.H. Suffet, Advances in Chemistry Series, Vol.202, (American Chemical Society, Washington D.C.) pp. 455-479.
3. Kool, H.J. and C.F. Van Kreijl, 1984. Formation and removal of mutagenic activity during drinking water preparation, Water Res. 18, 111-1016.
4. DeMarco, J., Stevens, A.A., and Hartman, D.J. 1981. Application of organic analysis for evaluation of granular activated carbon performance in drinking water treatment, in Advances in the Identification and Analysis of Organic Pollutants in Water, Ed., L.H. Keith (Ann Arbor, MI: Ann Arbor Science Publishers, Inc), pp. 907-940.
5. DeMarco, J., R. Miller, D. Davis and C. Cole. 1983. Experiences in operating a full scale granular activated-carbon system with on-site reactivation, in Treatment of Water by Granular Activated Carbon, Eds., M.J. McQuire and I.H. Suffet, Advances in Chemistry Series Vol. 202 (American Chemical Society, Washington, DC) pp. 525-563.
6. Loper, J.C., M.W. Tabor, L. Rosenblum and J. DeMarco. Continuous removal of both mutagens and mutagen forming potential by an experimental full scale granular activated carbon treatment system, Environ. Sci. Technol. in press, 1984.

7. Tabor, M.W. and J.C. Loper, 1980. Separation of mutagens from drinking water using coupled bioassay/analytical fractionation. Intern. J. Environ. Anal. Chem. 8, 197-215.

8. U.S. Environmental Protection Agency Office of Research and Development, 1980. IERL-RTP Procedures Manual: Level I Environmental Assessment 2nd Ed., Washington, D.C. USEPA-600/7-78-201 PB-293795.

9. LeBel, G.L., D.T. Williams, G. Griffith and F.M. Benoit, 1979. Isolation and concentration of organophosphorus pesticides from drinking water at the ng/l level using macroreticular resin. J. Assoc. Off. Anal. Chem. 62, 241-249.

10. Ames, B.N., J. McCann and E. Yamasaki. 1975. Methods for detecting carcinogens and mutagens with the Salmonella/mammalian-microsome mutagenicity test. Mutat. Res. 31, 347-364.

11. Kool, H.J., C.F. Van Kreijl and B.C.J. Zoeteman. 1982. Toxicology assessment of organic compounds in drinking water. CRC Critical Reviews in Environmental Control 12, 307-359.

12. Coleman, W.E., J.W. Munch, W.H. Kaylor, R.P. Steicher, H.P. Ringhand and J.R. Meier, 1984. Gas chromatography/mass spectroscopy analysis of mutagenic extracts of aqueous chlorinated humic acid. A comparision of the byproducts to drinking water contaminants. Environ. Sci. Technol. 18, 674-681.

13. Jolley, R.L. 1981. Concentrating organics in water for biological testing. Environ. Sci. Technol. 15, 874-880.

14. Monarca, S., J.R. Meier and R.J. Bull. 1983. Removal of mutagens from drinking water by granular activated carbon, Water Res. 17, 1015-1026.

LEGENDS TO FIGURES

Figure 1. Mutagenic activity recovered from carbon taken from different levels of granular activated carbon following its use in 35 weeks of water treatment. Open bars, assayed in the absence of microsomal activation (-S9); stippled bars, +S9. Reproduced from Loper et al, reference 6.

Figure 2. Schematic of the water plant. All of the mutagenic activity detected in water influent to the granular activated carbon (GAC) arose following the chlorination step carried out at the East Chemical Building, see Loper et al, reference 6.

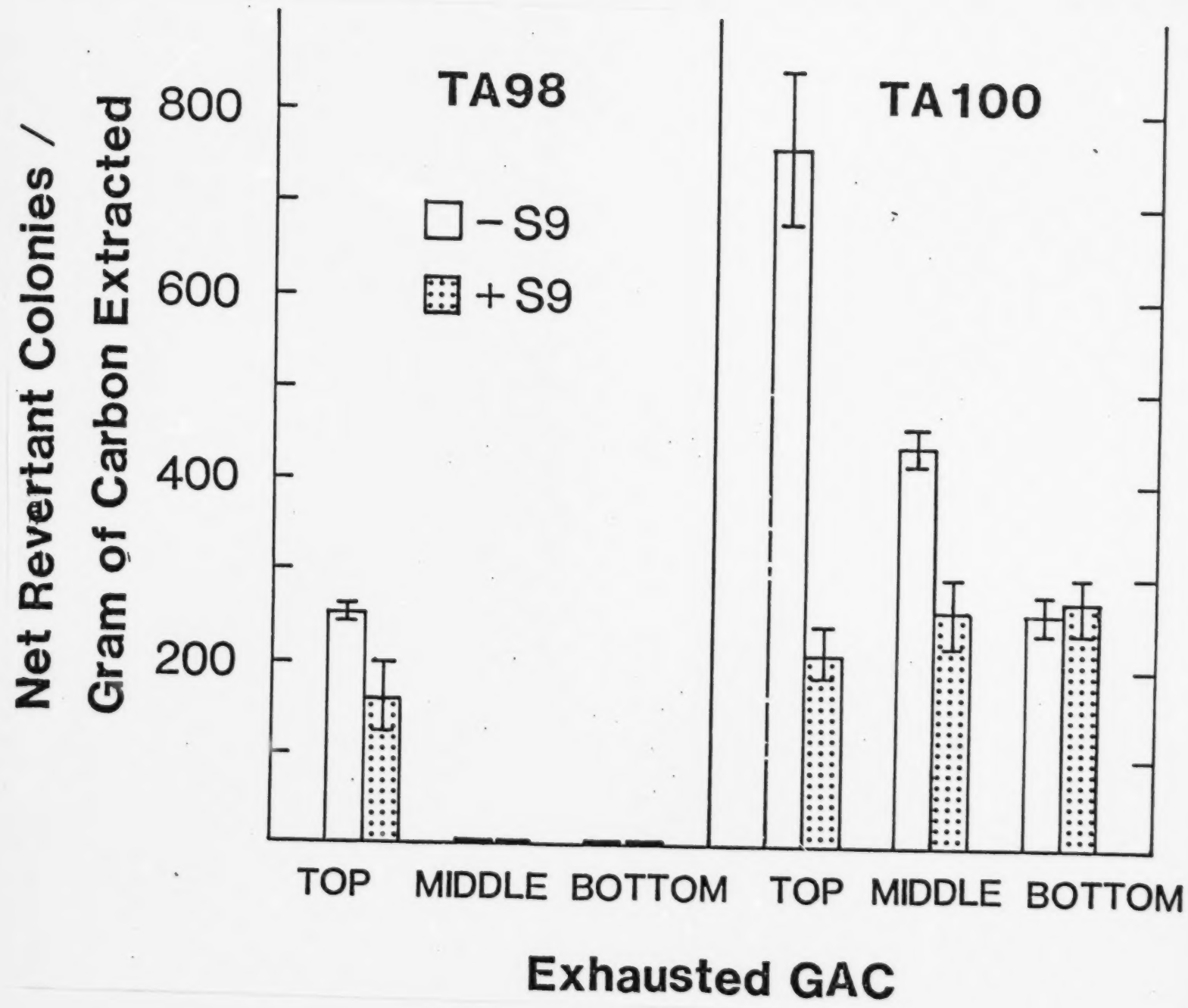
Figure 3. Absorption profiles by reverse phase HPLC of residues extracted from carbon samples removed from the same GAC absorber used for Figure 1. A: Residue from 350 mg of virgin carbon, and solvent blank. B: Residues from 35 mg and 23 mg of carbon of the used adsorber taken from the top and bottom, respectively. C: Residues of B were dissolved in water and reextracted with XAD-2.

Figure 4. Recovery by XAD of mutagenic residues originally isolated from the top and bottom of the used GAC adsorber. Activity of parent residue from top or bottom carbon is indicated by -■-. Activities from water solutions of these residues, reextracted by XAD are: XAD-2, -●-, XAD-7, -▲-.

Figure 5. Normal phase HPLC separation of residue organics extracted from used GAC. The separation was accomplished via a series of isocratic and linear gradient elutions using hexane, methylene chloride and methanol mobile phases, as described in the Materials and Methods section. Mutagenic activity was recovered in Fractions 1 and 5.

Table 1. Characteristics of granular activated carbon (GAC) adsorber.

GAC Data		Hydraulic data	
Manufacturer and type	Westvaco, WV-G	Diameter (ft)	11
Mesh size	12x40	Bed height (ft)	14.8
Total BET surface area (m^2g^{-1})	1100 mininum	Surface loading (gpm ft^{-2})	6.94
Mean particle diameter (mm)	0.90-1.20	Flow per day (mgd)	1
Carbon weight (lbs)	42,000	Residence time (min)	15


Net Revertant Colonies / Gram of Carbon Extracted
800
600
400
200
TA98
TA100
□ - S9
▨ + S9
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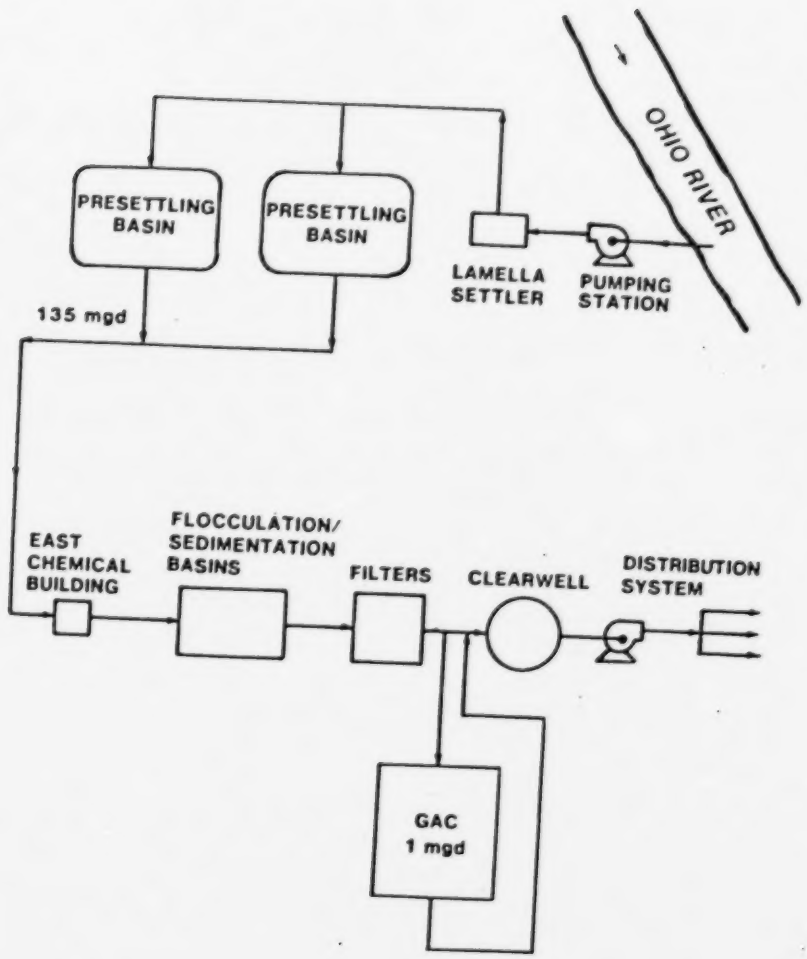
MIDDLE
BOTTOM
TOP
MIDDLE
BOTTOM
Exhausted GAC

Figure 1.

Figure 2.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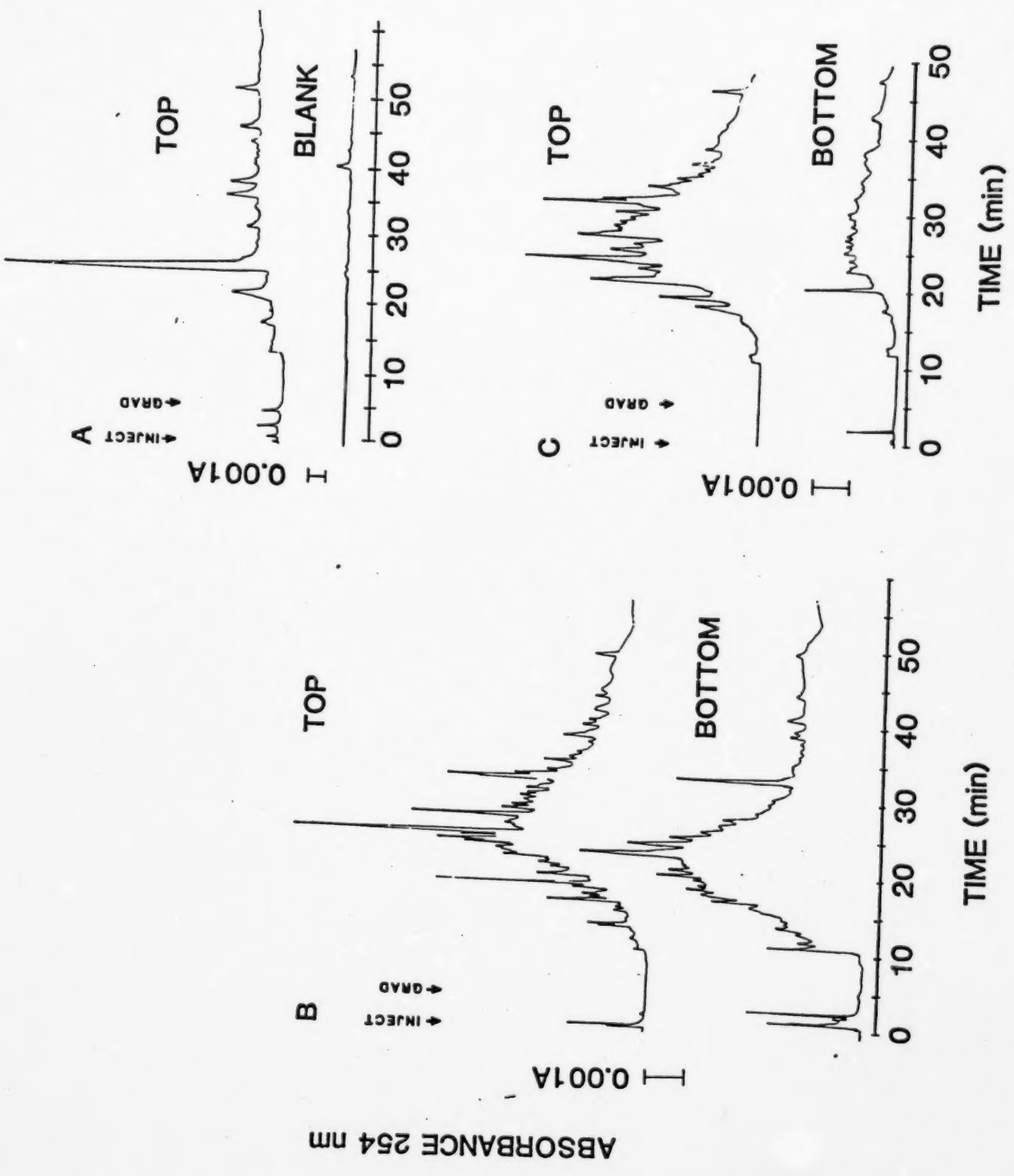
FILTERS
CLEARWELL
DISTRIBUTION SYSTEM
GAC 1 mgd

Figure 3.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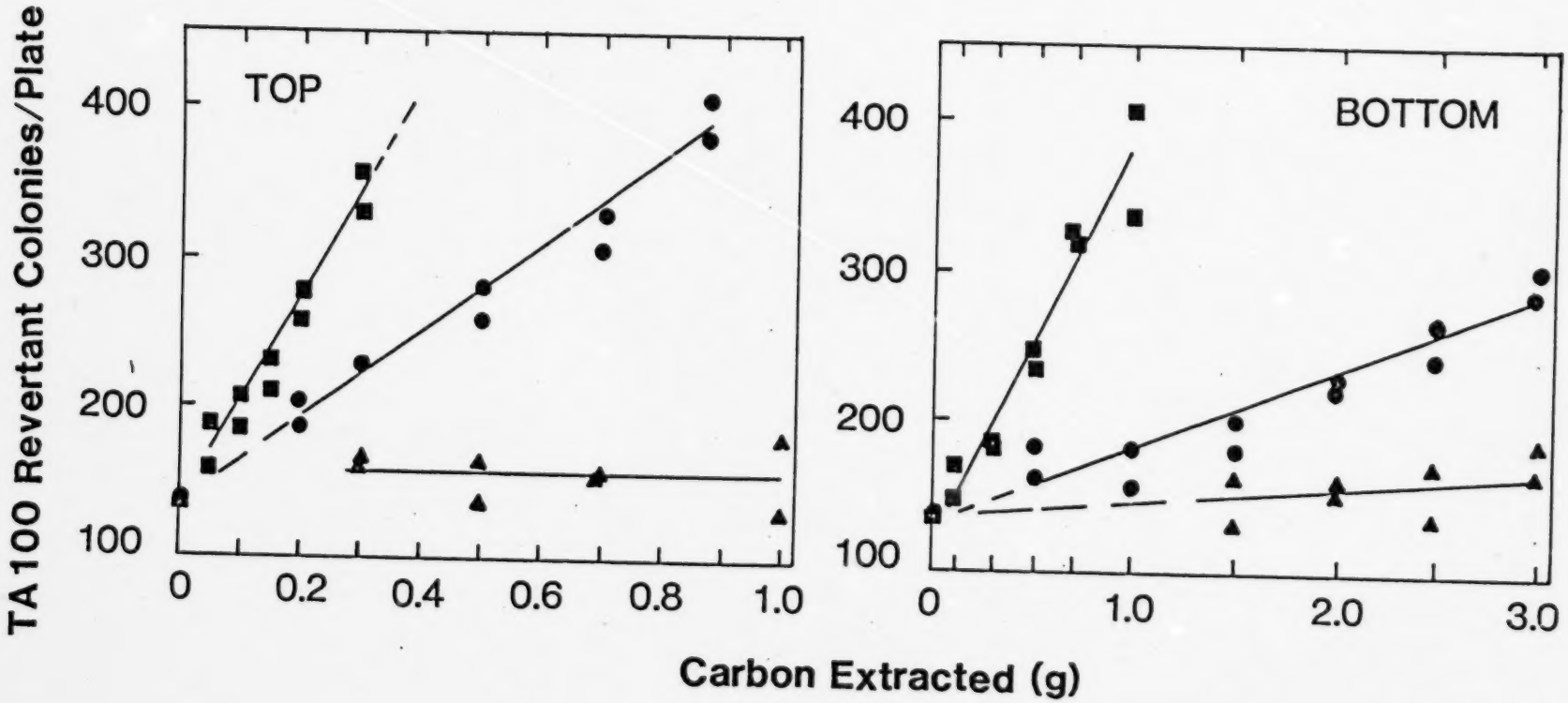
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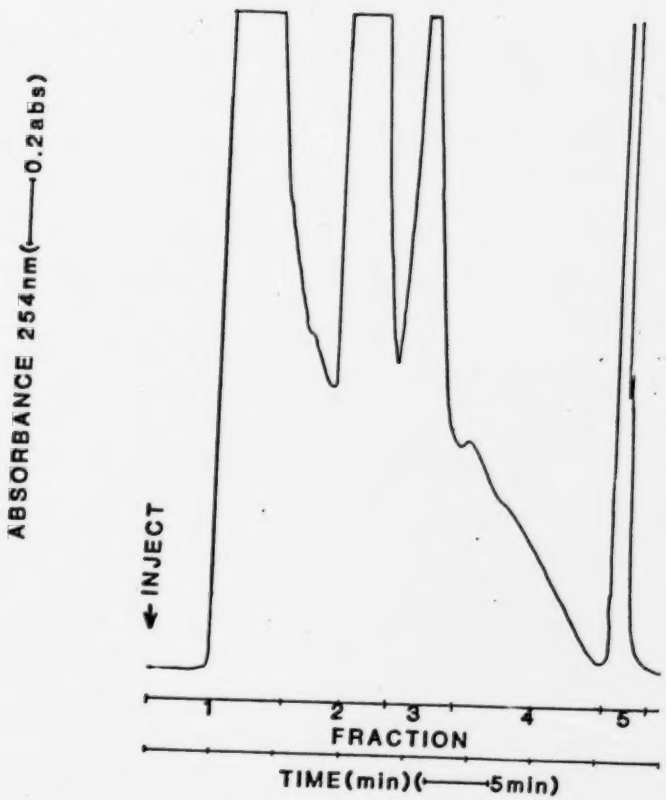
Carbon Extracted (g)

Figure 4.

Figure 5.


ABSORBANCE 254nm(——0.2abs)
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